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Ultrasonographic tools used in the evaluation of the canine spleen: A review¤

Herramientas ultrasonográficas usadas en la evaluación del bazo en caninos: Revisión de literatura

Ferramentas ultrassonográficas para avaliação do baço em cães: Revisão da literatura

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Abstract

The spleen is one of the most susceptible organs to primary tumours, metastasis, and a wide range of diseases of the hematopoietic system. Therefore, detailed assessment of the spleen is of great importance in veterinary medicine, especially in dogs, due to their economic and sentimental value, and as an experimental model in human medicine. Considering the recent advances in diagnostic imaging in companion animals, this review aims to describe the applicability of acoustic radiation force impulse (ARFI) elastography (qualitative and quantitative), Doppler, and contrast-enhanced ultrasonography for evaluating the spleen in dogs. The ARFI elastography is a recent ultrasound method that can provide reference values and aid in the diagnosis and evaluation of splenic abnormalities routinely encountered in veterinary practice. Conventional ultrasonography of the spleen combined with haemodynamic analysis by Doppler and contrast enhanced ultrasonography is an important tool in diagnosis and triage.

Keywords: *B-mode, contrast enhanced ultrasonography, dog spleen, Doppler, elastography.*

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Resumen

El bazo es uno de los órganos más propensos a desarrollar tumores, tanto primarios como metastásicos, y varias enfermedades que afectan el sistema hematopoyético. Es por eso que la evaluación clínica del mismo es de gran importancia en medicina veterinaria, principalmente en perros, debido a su valor económico, afectivo, y como modelo experimental en medicina humana. Considerando los recientes avances en imagenología diagnóstica, esta revisión tiene como objetivo describir el examen ecográfico del bazo en perros, utilizando la técnica de elastografía por impulso de fuerza de radiación acústica (ARFI) cuantitativa y cualitativa, Doppler y ultrasonido contrastado. La elastografía ARFI es un método reciente que puede proveer información básica sobre la conformación normal del órgano y, en un futuro próximo, ayudar en el diagnóstico de las enfermedades esplénicas. De modo similar, la ecografía convencional, Doppler y el ultrasonido contrastado son importantes herramientas en el diagnóstico y en el triaje.

Palabras clave: *bazo canino, Doppler, elastografía, modo-B, ultrasonografía contrastada.*

Resumo

O baço é um dos órgãos mais propensos a desenvolver tumores primários, metastáticos e afecções do sistema hematopoiético. Por isso, realizar uma avalição apurada neste órgão é de suma importância em medicina veterinária, particularmente em cães, devido à importância econômica, afetiva e de similaridade científica com o homem. Considerando os recentes avanços em diagnóstico por imagem, esta revisão visou descrever a aplicabilidade das novas técnicas ultrassonográficas para avaliação esplênica de caninos, utilizando a elastografia por impulso de força de radiação acústica *(*ARFI) qualitativa e quantitativa, Doppler e ultrassonografia por contraste com microbolhas. A elastografia ARFI é um método recente que pode fornecer dados de base para o baço e permitir a utilização futura desta técnica na avaliação de cães com doença esplênica. Similarmente, a ultrassonografia convencional, o método Doppler e a ultrassonografia contrastada são importantes ferramentas no diagnóstico e triagem.

Palavras chave: *baço canino, Doppler, elastografia, modo-B, ultrassonografia contrastada.*

Introduction

The spleen is the largest reticuloendothelial organ in mammals and one of the main sites for primary and metastatic tumours and diseases of the haematopoietic system (Morris and Dobson, 2007). The diagnosis of splenic alterations is of particular interest due to its involvement in lymphatic, immune, circulatory, and haematopoietic functions (Fry and McGavin, 2007).

Morphologic abnormalities of the spleen, including focal masses and diffuse alterations with or without overall splenic enlargement, are common in the dog (Couto, 2010). Nodular splenic disease is also common and can be caused by non-neoplastic (focal nodular hyperplasia, haematoma, infarct, or abscess) or neoplastic processes (haemangiosarcoma, lymphoma, malignant histiocytosis, or other mesenchymal tumours; Buergelt *et al*., 2001).

Ultrasonography is a useful imaging tool to evaluation of splenic abnormalities such as splenomegaly and changes in parenchyma echogenicity and echotexture (Feliciano *et al.*, 2015a). Focal splenic lesions are frequently identified during routine ultrasonographic examination, especially in older animals. These are often incidental findings and represent a diagnostic challenge (Mattoon and Nyland, 2015). However, conclusive diagnosis of various splenic pathologies can only be obtained by histopathological analysis of samples collected via an ultrasound-guided biopsy or after splenectomy (Rodaski and Piekarz, 2009).

Currently, there is an increasing interest in non-invasive diagnostic procedures using novel ultrasonography-based imaging modalities (Maronezi *et al*., 2015b). B-mode, Doppler, elastography [strain, shear wave, and acoustic radiation force impulse (ARFI)], and contrast-enhanced ultrasonography

can be used in splenic evaluation. B-mode and Doppler ultrasound provide real time information on the vascular architecture and hemodynamic aspects of the vessels (Carvalho *et al*., 2008). The ARFI elastography is safe, non-invasive, and provides quantitative and qualitative measures of tissue rigidity with reduced inter-observer variability (Dudea *et al.,* 2011; Feliciano *et al*., 2014a). Microbubble contrast-enhanced ultrasound increases the Doppler signal and detects blood flows that are hardly detectable by traditional methods (Nogueira *et al.,* 2002).

Considering the importance of these imaging techniques, this review aims to describe the main applicability of each ultrasonographic method in the evaluation of the canine spleen.

B-mode ultrasonography

Ultrasonographic examination of the splenic parenchyma in dogs requires clipping the abdominal hair to facilitate transcutaneous ultrasonography (Feliciano *et al.,* 2007). The procedure should be performed with the animal in fasting state, following antiphysetics administration (Feliciano *et al.,* 2015a). Dorsal or lateral recumbence may be used, and curvilinear, convex or linear transducer (5 to 10 MHz frequency) is routinely used, depending on the size of the animal. The long axis of the spleen has a tongue-like shape and the cross-section a triangular or lenticular form. The cranial aspect is in dorsal location and visible between the gastric fundus and the left kidney (Hecht and Mai, 2015). In dogs, the splenic parenchyma has a homogeneous echotexture and is covered by a thin and very hyperechoic capsule. It is generally hyperechoic to the liver and renal cortices (Matton and Nyland 2015). Branches of the splenic vein appear as tubular anechoic structures within the splenic parenchyma and can be seen leaving the spleen at the hilus. Splenic arteries, on the other hand, are usually not visible unless Colour-Doppler imaging is used (Hecht and Mai, 2015).

Ultrasonographic examination of the spleen is clinically useful to determine the location, size, and presence of parenchymal abnormalities when pathological conditions are suspected. The main indications for ultrasonographic evaluation of the spleen are generalized splenomegaly, abdominal or splenic mass, trauma, and hemoperitoneum (Matton and Nyland, 2015).

The ultrasonographic assessment of splenic size is subjective. In dogs with splenomegaly, the borders of the spleen appear rounded and the organ extends caudally and to the right side of the abdomen (Hecht and Mai, 2015). Splenomegaly with homogeneous echotexture is a frequent finding in dogs sedated with acepromazine, thiopental (O'Brien *et al*., 2004), ketamine, and diazepam (Wilson *et al.,* 2004). The maximum thickness of a normal spleen before anaesthesia (acepromazine, thiopental) is 1.7 cm. Splenic enlargement is observed approximately 15 min after acepromazine administration, resulting in a maximum splenic thickness of 2.6 cm (Wilson *et al.,* 2004)

Ultrasonography has proven to be highly sensitive to detect focal parenchymal abnormalities in dogs. However, a large overlap exists between the ultrasonographic appearance and the aetiology of lesions and, thus, the ultrasonographic appearance of an individual lesion is rarely, if ever, pathognomonic of the underlying disease. Therefore, it is very difficult to confidently differentiate benign from malignant nodules based on ultrasonographic appearance alone, and thus tissue sampling is usually required for the diagnosis (Warren-Smith, 2012). Fine needle aspiration and Tru-cut needle biopsy are commonly used to differentiate between benign and malignant lesions. However, these minimally invasive techniques generally require sedation or general anaesthesia (Matton and Nyland, 2015).

Doppler ultrasonography

Among the recent advances in ultrasound technology, Doppler ultrasonography has achieved prominence for being able to assess the function of various organs (Carrillo *et al.,* 2012). Triplex Doppler ultrasound involves the simultaneous use of two-dimensional ultrasonography, colour Doppler, and pulsed wave Doppler. It is used to obtain anatomical data of vessels and functional data of blood flow, such as presence or absence, direction and speed of flow, making it the method of choice for assessing the vascularity of several organs, including the spleen (Pozor and McDonnell, 2004; Wood *et al.,* 2010; Souza *et al.,* 2014).

The splenic vasculature consists of the splenic artery and the splenic vein. The arterial branches are difficult to see during routine scans, but Doppler ultrasonography can help determine their location and patency. In healthy dogs, the branches of the splenic vein can be easily visualized near the hilum of the spleen, but their course into the splenic parenchyma can be followed only for a short distance (Matton and Nyland, 2015).

The use of Doppler ultrasonography in animals is recent and has shown promising results. It is effective for the hemodynamic evaluation of tissues and is particularly useful in the analysis of tissue neovascularization in splenic disorders, neoplasia, and torsion; the latter being characterized by the absence of blood flow (Gil *et al.,* 2015). Furthermore, Doppler ultrasonography is useful in diagnosing splenic vein thrombosis in abdominal inflammatory disease or other pathologic conditions that could lead to hypercoagulability. It is also the procedure of choice for distinguishing splenic congestion and inflammation from severe vascular compromise caused by splenic torsion or vascular thrombosis (Matton and Nyland, 2015).

Colour and power Doppler evaluation of blood flow in splenic masses do not allow differentiating between benign and malignant cases in dogs. However, the presence of a wide or tortuous vessel within the mass is suggestive of malignancy, as is the peritoneal effusion (Sharpley, 2012).

The main splenic characteristics evaluated by spectral Doppler ultrasonography are the presence or absence of neovascularization and the calculation of vascular indices: peak systolic velocity (PSV), end-diastolic velocity (EDV), and resistance index (RI = [PSV - EDV] / PSV) of the splenic artery (Feliciano *et al.,* 2013). These indices have an advantage over flow velocity colour Doppler measurements, which are independent of the angle of insonation (Nelson and Pretorius, 1988; Middleton *et al.,* 1989; Gumbsch *et al.*, 2002; Carvalho *et al.,* 2008; Figure 1).

Evaluation of splenic blood flow by Doppler often reveals low flow resistivity with broad and continuous peak systolic flow, increased diastolic peak flow or full diastole, and peak systolic flow without a spectral window, similar to the spectral pattern of hepatic and renal arteries (Gil *et al.*, 2015).

Information of the Doppler technique is limited to the characterization of the splenic vascularization using pulsed Doppler tracing (Albernaz *et al.,* 2007; Santarém *et al.,* 2008). The reported vascular

Figure 1. Colour and spectral Doppler ultrasonography image of the splenic parenchyma in a healthy dog. Note the colour-Doppler identifying a branch of the splenic artery (red) and satellite veins (blue), the spectral gate localization (above) and the assessments of vascular indices (below and right crosses).

indices of the splenic artery in dogs with subclinical ehrlichiosis are PSV of 22.59 ± 8.07 cm/s, EDV of 5.25 ± 4.66 cm/s, and RI of 0.71 ± 0.14. These vascular indices are important in animals infected with haemoparasites and can be useful in the therapeutic monitoring of patients that lack clinical signs. It is important to mention that splenic artery Doppler scanning may be difficult due to respiratory and intentional movements of the patient, which hinder the tracing and prolong the examination time (Maronezi *et al.,* 2015a).

Elastography

Currently, there is an increasing interest in the non-invasive assessment of viscoelastic properties of internal organs and tissues with novel ultrasonography-based imaging modalities (Maronezi *et al*., 2015b). In human medicine, new imaging techniques, such as elastography, have been developed. This ultrasound method is the technical evolution of one of the oldest medical tools, palpation; and thus evaluates the shape and stiffness of the organ of interest (Ophir, 2002).

To evaluate tissue stiffness, several methods have been proposed and grouped as elastographic techniques, among them stands out: Strain rate by manual compression, acoustic radiation force impulse (ARFI), and real-time shear velocity (RSV; Dudea *et al*., 2011). Additionally, tissue hardness can be categorized qualitatively (e.g. strain rate, strain intensity) or quantitatively (e.g. shear wave velocity; Feliciano *et al*., 2015a).

In qualitative assessment, the tissue is subjected to a force (automatic or manual) and the image software measures tissue response to this force, creating an image known as elastogram (black and white or colourful scales). On applied form, the harshest tissue deforms less by compression and is classified in the scale as hard, while the less stiff has greater deformation and classifies as soft (Feliciano *et al*., 2015a). These techniques are able to detect both movements, compression and extension, of the tissue along the axis. It can be performed in real time or off-line, but in clinical practice are commonly applied in real time. Ultrasound requires a specific software adapted for evaluating the physical properties of a tissue, determining its relative degree of hardness compared with adjacent tissues through local compression. (Carvalho *et al*., 2013).

The Compression elastography technique applies a manual or automatic mechanical pressure with the transducer, and the software measures the resulting deformation. This technique is based on tissue tension estimated by the time "delay" between ultrasound signals during pre and post-compression. The stress caused by compression typically changes according to depth (i.e. stress and elasticity decrease when depth increases). Thus, tissue depth becomes a limiting factor for this technique, particularly for assessing deep organs or obese patients (Carvalho *et al*., 2015; Feliciano *et al*., 2015a). Furthermore, in this technique the acquisition and interpretation of elastography image data is largely dependent on the experience of the examiner (Burnside *et al*., 2007; Chang *et al*., 2011). As an advantage, this technique is commercially available, and it has shown promising results and clinical utility in human medicine (Carvalho *et al*., 2015; Feliciano *et al*., 2015a).

The ARFI elastography is an imaging technique that provides tissue stiffness by means of qualitative and quantitative measurements with a reduced inter-observer variability (Feliciano *et al*., 2015b). In qualitative ARFI evaluation, short acoustic pulses at high intensity are used to deform tissue elements and create a static greyscale map (elastogram) of the relative tissue stiffness. Generally, lighter areas represent more pliable tissues (Goddi *et al.,* 2012; Figure 2). Using this technique, measurements are more objective, because it avoids the variation of compression force applied by the operator. Thus, the method becomes more accurate with less inter-observer variation and greater reproducibility (Carvalho *et al*., 2013).

The elastography quantitative analysis can be performed by two different methods that require specific equipment and software: ARFI elastography imaging and supersonic shear wave imaging. In quantitative ARFI, the primary acoustic impulse directed towards the region of interest (ROI) promotes forming pressure waves capable of deforming the tissue, thus increasing the velocity of wave propagation (shear velocity). Wave velocity and the attenuation of acoustic pressure waves are related

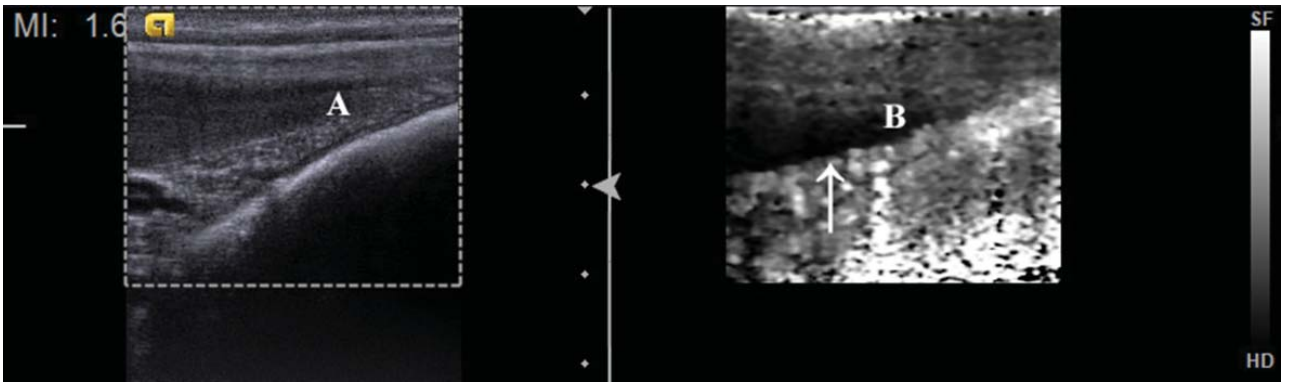

Figure 2. Ultrasonographic image of the spleen in a healthy dog during qualitative acoustic radiation force impulse (ARFI) evaluation. (A) B-mode image of the tail of the spleen, (B) elastographic analysis of the spleen area (arrow) with characterization of the stiffness of the splenic tissue in greyscale and evaluation of the deformity.

to tissue stiffness and viscoelasticity (Comstock, 2011; Figure 3). The shear wave velocity obtained by this method will be faster in hard tissues than in soft tissues. Also, the software may provide a color coded image displaying the shear wave velocity (m/sec) or elasticity (kPa) for each defined ROI and a variety of stiffness parameters may be measured, including the mean, maximum, and standard deviation of stiffness (Sebag *et al*., 2010; Bhatia *et al*., 2012). The ARFI elastography has several advantages over other techniques; this evaluation can be combined with routine real time ultrasound and moreover, due to its greater penetration compared with mechanical compression, this technique is of great help in ascites and obese patients. However, the high cost of the equipment and training for ARFI limits its clinical application (Carvalho *et al*., 2013).

In veterinary medicine, the ARFI technique is a recent development and has been used to evaluate focal liver lesions in rats (Carvalho *et al.,* 2012), mammary tumours in bitches (Feliciano *et al.,* 2014a), and queens (Feliciano *et al*., 2015c), prostate and testes in dogs (Feliciano *et al.,* 2015b), kidneys (Garcia *et al,* 2015), testes (Brito *et al*., 2015), and spleen in cats (Feliciano *et al.,* 2014b), spleen, liver, and kidneys in adults dogs (Holdsworth *et al.*, 2014), and spleen in healthy dogs of different age (Maronezi *et al.*, 2015b).

The manual compression elastography and ARFI techniques were related for evaluating canine splenic stiffness (Holdsworth *et al*., 2014; Jeon *et al.*, 2015; Maronezi *et al*., 2015b). Qualitative evaluation reported by Jeon *et al*. (2015) was performed on the colour elastogram and the calculated splenic strain intensity was compared with abdominal wall strain intensity, resulting in the splenic strain rate that may be useful for normal and abnormal spleen evaluation. A preliminary study published by Holdsworth *et al*. (2014) determined the quantitative elastographic characteristics of the normal liver, spleen, and kidneys in 15 healthy dogs at predetermined depths within the parenchyma. The values obtained for the spleen were 1.59 to 2.4 m/s at 0 to 2 cm deep and 1.45 to 1.94 m/s at 2 to 4 cm. They observed that depth had a significant negative correlation with shear wave velocity. Furthermore, Maronezi *et al*. (2015) examined healthy splenic segments by qualitative elastography in dogs and related normal mean shear velocity values for the head (2.32 m/s), body (2.16 m/s), and tail (2.25 m/s) of the spleen, and did not find differences between age groups (Maronezi *et al.,* 2015b).

Quantitative and qualitative ARFI elastography of the spleen in dogs is easily performed and may provide additional criteria for the evaluation and diagnosis of splenic abnormalities typically obtained by B-mode ultrasonography. However, some patients may not cooperate, move and disrupt the acquisition of measurements. In these cases sedation may be necessary to obtain an appropriate assessment. Researchers suggest keeping subjects in a quiet environment with minimum restraint (Feliciano *et al.,* 2014b; Garcia *et al.,* 2015).

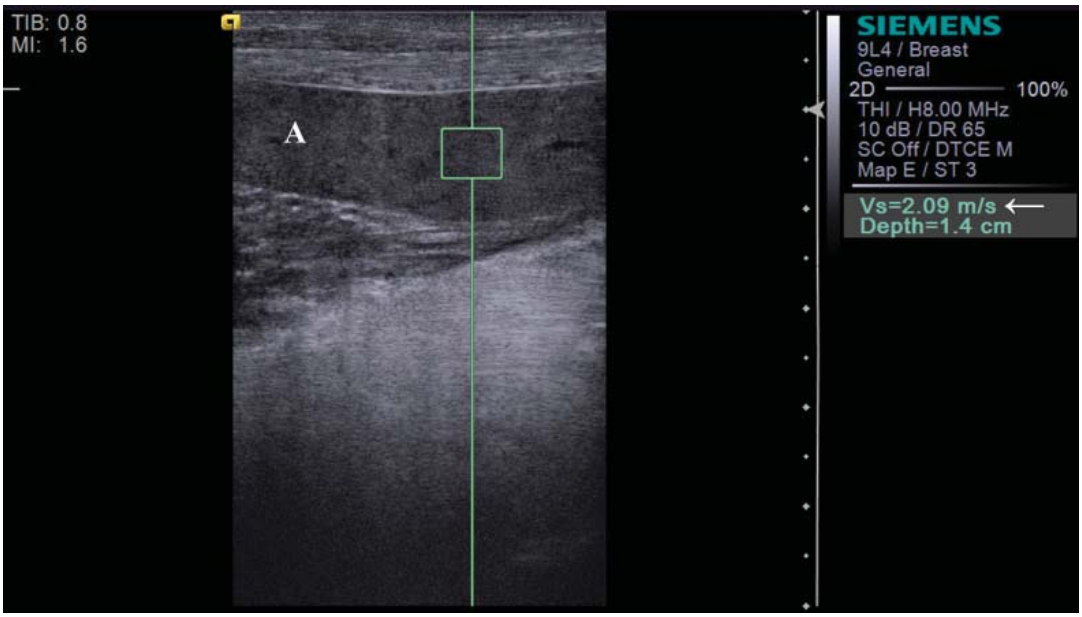

Figure 3. Ultrasonographic image of the spleen body in a healthy dog (A) subjected to quantitative acoustic radiation force impulse (ARFI) evaluation. A gate (green box) was used to select a region of the splenic tissue for shear wave velocity determination (m/s). The region of interest box has been placed within the superficial portion of the spleen at a depth of 1.4 cm (from the skin-transducer interface).

Contrast-enhanced ultrasonography

Contrast-enhanced ultrasonography (CEUS) is an innovative method that improves detection of perfusion and vascularity of organs (Nyman *et al.*, 2005). Ultrasound contrast agents are tiny bubbles of gas that are too small to be caught in the capillary bed, but sufficiently large to enhance the reflection of flowing blood. High molecular weight gases, such as perfluorocarbons, are used for the microbubbles. Microbubbles measure between 1 and 6 µm in diameter, a size that enables free passage through all capillary beds. The advantage of ultrasound contrast agents vs contrast agents used in magnetic resonance or computed tomography is that the bubbles behave in the same way as red blood cells and do not diffuse out of the vascular space (Kalantarinia and Okusa, 2007).

Ultrasound waves directed at the vasculature are scattered upon interaction with intravenously-infused contrast agents, resulting in a bright image of the vasculature. Therefore, using contrast agents with ultrasound enables detecting microcirculation changes, improves Doppler imaging in tissues with slow blood flow rates, and provides more detailed information on vasculature structure during imaging in B-mode. Ultrasound contrast agents have proven to be safe and free of hemodynamic effects, making them ideal for the study of tissue blood flow, micro and macrovascular flow patterns, velocity and perfusion (Lindner, 2002). Ultrasonography evaluation with contrast agent takes about 20 min (Haers and Saunders, 2009).

Microbubble contrasted ultrasound has been considered in some cases to be superior to magnetic resonance imaging and computerized tomography (contrasted) in the assessment of focal nodules in parenchymal organs in humans. Furthermore, its use does not require anaesthesia, there is no radiation emission, and it provides real-time images (Haers and Saunders, 2009). In humans, this technique has been used in testicular diseases to obtain higher diagnostic accuracy when ultrasound findings are inconclusive (Souza *et al.,* 2014).

Microbubble contrasted ultrasound is a new technique in veterinary medicine and has been used to evaluate liver (O'Brien, 2007), spleen (Ohlerth *et al*., 2007), abnormal spleen (Ivancic-Arndt *et al.,*

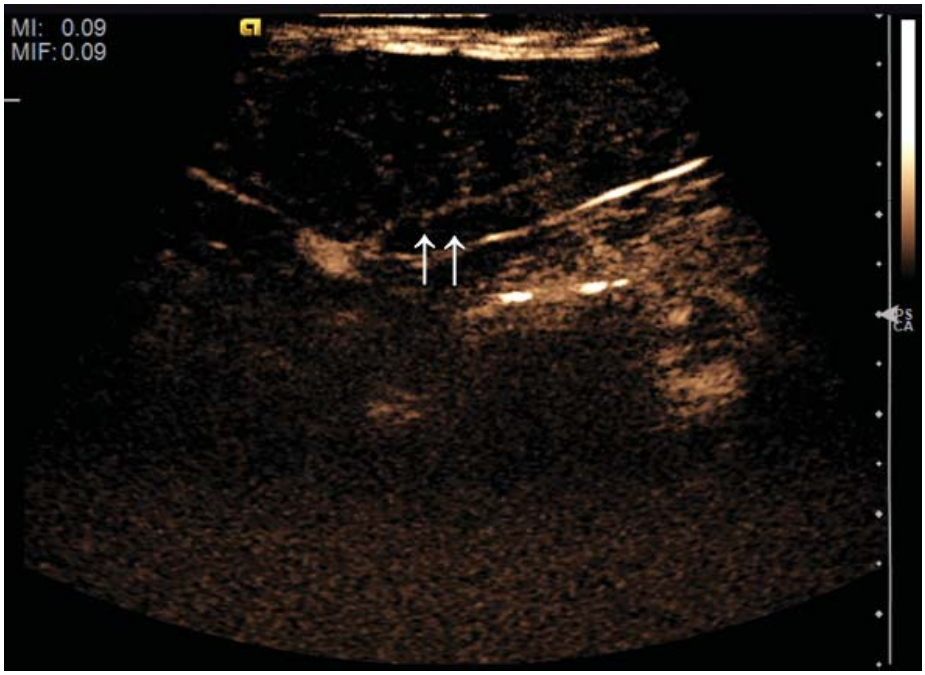

Figure 4. Ultrasonographic image of the spleen in a healthy dog using contrast-enhanced ultrasonography (arrows). The homogeneous splenic parenchyma can be visualized in the peak enhancement phase.

2007; Maronezi *et al.,* 2015a), kidneys (Waller *et al*., 2007), pancreas (Rademacher *et al.,* 2015), lymph nodes (Schärz *et al.,* 2005), and portosystemic shunts in dogs (Salwei *et al.,* 2003).

The information obtained by contrast examination enables to establish parameters related to homogeneous or heterogeneous tissue filling (Volta *et al.,* 2014). Furthermore, in conjunction with microbubble contrast-enhanced ultrasonography, B-mode is used to determine wash-in, wash-out and peak enhancement time of the contrast in the tissue, which were correlated with tissular perfusion (Takeda *et al.,* 2012). The microvascular fill pattern of contrast-enhanced ultrasonography enables detecting small masses at early stages of evolution and hypervascularization in aggressive tumours, aiding in non-invasive differentiation of malignant and benign tumours (Lock *et al.,* 2009).

Contrast-enhanced ultrasonography of the spleen revealed mean wash-in time in healthy dogs to be 13.2 s and peak enhancement time 29.8 ± 11.6 s (Ohlerth *et al.,* 2007; Figure 4). In humans, wash-in time is 12.0 s and approximately 50 s after contrast injection the splenic parenchyma becomes homogeneous (peak enhancement) and a dense enhancement persists for 5 to 7 min (wash out; Catalano *et al.*, 2004).

Contrast-enhanced ultrasonography evaluation of the spleen in dogs with subclinical ehrlichiosis revealed wash-in time of 5.31 ± 0.7 s, peak enhancement time of 18.56 ± 2.90 s, and wash-out time of 94.56 ± 35.21 s; which were lower than those reported in the literature (Maronezi *et al.,* 2015a; Figure 5).

Non-invasiveness, no anaesthesia, and real-time evaluation are the main advantages of contrast-enhanced ultrasonography. The disadvantages are the equipment requirements and high cost of ultrasound contrast agents. Additionally, pulmonary contrast elimination may limit its use in patients with pulmonary diseases. Some adverse effects of contrast were observed in humans (headaches, diarrhea, neutropenia, nausea, skin reactions, dyspnoea, rhinorrhagia), but not in small animals (Wdowiak *et al*., 2010). In experimental studies with sheep, dexamethasone was used to prevent pseudo-anaphylaxis and pulmonary hypertension due to acute and transient reactions (right ventricular dilatation and

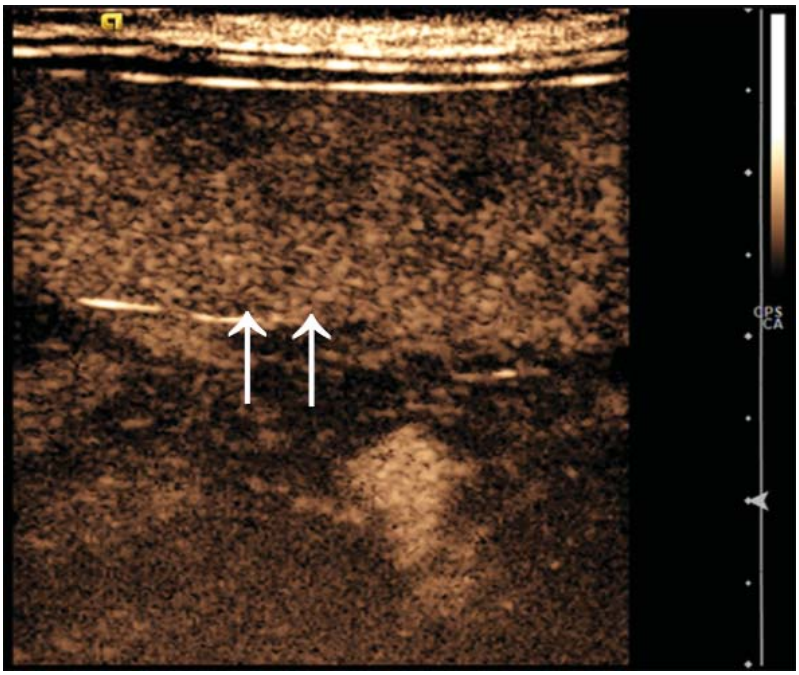

Figure 5. Ultrasonographic image of the spleen in a dog with subclinical ehrlichiosis using contrast-enhanced ultrasonography. Note the contrast-enhanced ultrasonography with mixed echogenicity and heterogeneous echotexture (arrows) in the peak enhancement phase.

pulmonary pressure increase) reported in pigs after contrast administration (Antoine *et al.,* 2014).

In conclusion, this review provides information on the ultrasonographic methods used for splenic exam in dogs. Conventional ultrasonography is useful for providing information on splenic parenchyma while Doppler is emerging as an important and suitable non-invasive tool for blood flow assessment. The ARFI elastography of the spleen in dogs may provide valuable criteria for the evaluation and diagnosis of splenic abnormalities, and the specificity of this imaging technique could aid differentiating malignant splenic neoplasias. Contrast-enhanced ultrasonography can also contribute to evaluate changes in the splenic tissue, perfusion and malignant differentiation.

Conflicts of interest

The authors declare that they are not financially or personally related to any individual or organisation that could inappropriately influence or bias the content of this article.

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